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AUTHOR(S):

OBATA, KAZUO

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Attitude of Trypsin Inhibitor at Acute Pancreatitis

by

KAZUO OBATA

2nd Department of Surgery, Kanazawa University, Medical School
(Director : Prof. Dr. ICHIO HONJO)
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CONTENT

I. INTRODUCTION

Acute hemorrhagic pancreatitis (abbreviated to acute pancreatitis, hereafter) is one of the characteristic diseases as featured by its fulminant clinical picture and unfavorable prognosis. As a cause of this fact, intoxication due to liberation of pancreatic enzymes has been emphasized. Among these enzymes, amylase is proved to increase in blood corresponding to the development of the disease. However, as to lipase and trypsin, particularly the latter, conspicuous increase is not demonstrated partly due to the difficulty of

determination until recent years.

On the other hand, it has attracted interest of many investigators since HILDEBRANDT's report in 1893 that there exists antitryptic activity in serum. Moreover, in 1948 KUNITZ[28] extracted some substance from the pancreas which inhibits the activity of trypsin and clarified its physical and chemical characters, which was then followed by successive discoveries of the substances having the same action within animal's tissue, plants of some kinds and more primitive animals and characters of these substances have gradually been disclosed up to present[48][18][19][13][16][6][7][24][36][53][44]. Today, these substances are commonly called *trypsin inhibitor* or *antitrypsin*[55][30]. Essential and character of each of these substances, however, are much different from each other, and details in this respect are not yet clarified.

Pancreatic trypsin inhibitor (abbreviated to P. T. I., hereafter) as reported by KUNITZ normally exists in pancreatic juice and inhibits simultaneously elaborated trypsinogen to be activated to trypsin within the pancreatic parenchyma or pancreatic duct, or it has an effect to combine itself with once activated trypsin to inactivate it[57]. As pancreatic juice appears in the duodenum, prokinase coexisting in the pancreatic juice is activated to enterokinase, which comes to surpass inhibitory action of P. T. I. and produces active trypsin. Protein digesting action of trypsin finally works here[59][26]. At acute pancreatitis, however, the process is somewhat different, considerable amount of active trypsin being found within the pancreas, and it is considered that as it is liberated from the pancreas[51], P. T. I. also comes to appear in blood stream, surrounding tissue and peritoneal cavity.

Character, mode of reaction and behavior at the diseased state of antitryptic properties in serum have been widely investigated by many researchers. As to the location of this property, LANDSTEINER maintained in 1900 its existence in albumin fraction by means of salt fractionation, which was thereafter followed by many approvals. In 1953, however, PEANASKY and LASKOWSKI[41] isolated trypsin inhibitor from α_1-globlin fraction. In 1955, SHULMAN[46] further isolated another trypsin inhibitor from α_2-globlin fraction. These results were ascertained by means of electrophoresis by JACOBSSON[24] in the same year. Recently, DYCE and HAVERBACK[14] also reported the same results. GROB[15] has identified the location of trypsin inhibitor using COHN's fractionation method.

In addition to these, KAZAL et al[27]. have demonstrated the existence of another inhibitor, so-called KAZAL's inhibitor or second inhibitor, which is different from that discovered by KUNITZ. Moreover, there are reports of discovery of inhibitor in urine[15] or colostrum[32], but it is not clarified whether or not this inhibitor is identical to above described serum inhibitor.

As a cause of the phenomenon that increase in serum trypsin is not so prominent at acute pancreatitis, mechanism of trypsin inactivation by these trypsin inhibitors can be inferred. Following experiments were attempted in order to ascertain this possibility, and in order to clarify complicated pathophysiology which may justifiably be called self-defence reaction and further to disclose the significance of this process in acute pancreatitis by clarifying activity relationship between trypsin inhibitor and liberated trypsin from the pancreas.

It is accepted generally that trypsin and trypsin inhibitor combine with each other

forming inactivated compound, which is enzymodynamically represented as follows;

$T+I \rightleftarrows T \cdot I$, whereby T represents trypsin, I trypsin inhibitor and T • I trypsin-trypsin inhibitor compound.

It is also said that in this formula the velocity of the reaction is maintained constant depending upon the conditions of the reaction, consequently above mentioned three components being held in equilibrium[23)40)]. At acute pancreatitis also liberated trypsin and trypsin inhibitor are considered to form coexistence of three components of active trypsin, active trypsin inhibitor and inactivated compound in a certain proportion. Although active trypsin and active trypsin inhibitor can be determined, amount of trypsin-trypsin inhibitor compound and amount or activity of each of trypsin and trypsin inhibitor which form the compound cannot be properly determined at present with some exception. On the other hand, inhibitory activity is presumed to be consisted of various trypsin inhibitors within the material, and there is no suitable method of respective determination of these inhibitors. Hence in the present experiment, active trypsin, active trypsin inhibitor and P. T. I., which is the sum of inhibitor of active form and combined form, are respectively determined. At this point, such reaction between trypsin and trypsin inhibitor gives an impression that the reaction well resembles antigen-antibody reaction in immunology. Little is known, however, concerning the essential and mutual relationship of these reactions, today. Accordingly, in the present experiment study was not carried out in the immunological aspect.

In the present paper, activity of inhibitor in materials were represented in the term of antitryptic activity (abbreviated to A. T. A.).

II. MATERIALS AND METHODS

1. Animals: Adult mongrel dogs weighing approximately 10 kg were used.

2. Production of Acute Pancreatitis: The abdominal cavity was opened under intravenous anesthesia of ISOZOL. Bile was aspirated by gall bladder puncture with a small needle. The main pancreatic duct was exposed and isolated from the surrounding tissues near its duodenal opening and the bile of 0.3 ml per kg body weight was injected into the main pancreatic duct under large pressure. The main duct was ligated with silk-thread, lest the injected bile should flow out into the intestine. Postoperatively, administration of antibiotics and fluid transfusion were withheld since a possibility of trypsin inhibiting effect of antibiotics and that of influence of fluid transfusion upon the amount of peritoneal fluid were suspected. It was also ascertained that ISOZOL has no interference on experimental results by the comparative determination performed before and after the injection of this agent.

3. Collection of Serum and Peritoneal Fluid: Blood of several ml was taken from the femoral vein certain period of time before and after the operation. Serum was separated immediately and subjected to the determination. Peritoneal fluid was collected through a small rubber tube inserted from an incision in the right hypochondrial region, one end being placed behind the duodenum slightly directed below. Another end of the tube was closed with a clamp, which was released when in use.

4. Preparation of Tissue Specimen: Tissue sections of about 1 g were taken, when necessary, by double ligation, laparotomy being carried out certain interval of time after

the production of acute pancreatitis.

5. False Laparotomy : As in the production of acute pancreatitis, the abdomen was opened, and the operation was performed merely to the extent to aspirate bile and expose the main pancreatic duct.

6. Determination of Trypsin Activity : Method of NARDI[37)38)39)] was followed, which is summarized as follows.

i. Material is heated at 65°C for 15 minutes.

ii. Buffer solution (phosphate buffer solution N/15, pH 7.8) of 0.5 ml and benzoyl-l-arginine amide hydrochloride monohydrate (abbreviated to B. A. A., hereafter) substrate solution of 1.0 ml are added to 0.5 ml of the material. The mixture is incubated at 25°C for an hour being shaken.

iii. On the other hand, 1.0 ml of boric acid solution and 2 drops of indicating reagent are put in the inner chamber of the CONWAY's unit, which is then tightly closed with a glass cover sealed with vaseline.

iv. An hour later, 0.2 ml of the mixture in above described ii. is poured in the outer chamber of the CONWAY's unit.

v. Immediately, 1.0 ml of saturated K_2CO_3 solution is pipetted in the opposite side of the outer chamber carefully so that the solution may not mix together with previously poured mixture of the material.

vi. The chamber is tightly closed with a cover and rotated 15 times, which is then let alone for an hour.

vii. An hour later, the material is titrated with hydrochloric acid of 0.01 N using micro-biurette until the change in color of boric acid caused by transference of pH returns to that of blank material, and consumption of hydrochloric acid is read.

viii. As the blank, 0.5 ml of buffer solution was used instead of the material with the identical procedures. Solution of crystalline trypsin (Tripure ; Novo Industry) of previously known activity was determined by this method, and trypsin activity which required 1μl of hydrochloric acid at titration was calculated and average value of 0.705×10^{-4} ANSON units was obtained. Activity of trypsin was represented in the term of ANSON unit (abbreviated to A. U., hereafter) which was converted from the amount of hydrochloric acid consumed at titration. As there exists ammonia of free type in peritoneal fluid, the value obtained from the determination with the material added with buffer alone insted of B. A. A. was substracted from the actually determined value.

7. Determination of P. T. I. : P. T. I. was extracted following the method of KALSER-GROSSMAN[26)], and subjected to the determination of hemoglobin method of ANSON[1)2)]. Results were represented in the term of A. U./ml. Procedures of the determination are summarized as follows.

a) Extraction

i. Trichlor acetic acid of 5 per cent solution of 2.0 ml is added to the same amount of tested material.

ii. The mixture is heated in a water bath of 80°C for 5 minutes.

iii. It is then centrifuged for 5 minutes at 2000 r. p. m. and filtrated.

iv. The supernatant is extracted with ether of the same amount for two times and

remaining ether is evaporated under partial vacuum.

v. The material is adjusted to pH 7.0 with 0.05 N NaOH using B. T. B. as indicating reagent.

b) Binding

Thus adjusted material of 0.5 ml is mixed together with 0.5 ml of diluted trypsin solution and kept at 5°C for 30 minutes.

c) Digestion

i. Thirty minutes later, 5.0 ml of hemoglobin substrate solution is added to the material and well stirred, which is incubated at 25°C for 10 minutes.

ii. Accurately 10 minutes later, 10 ml of 0.3 N trichlor acetic acid is added to the material and adequately mixed to interrupt digestive process and, at the same time, let stand for 30 minutes to precipitate surplus protein.

iii. The precipitate is filtrated through a hard filter paper (Toyo-roshi, No. 131).

d) Development of Color

i. Ten ml of 0.5 N NaOH is added to 5.0 ml of the filtrate and 3.0 ml of diluted phenol reagent is gently dropped to develop the color, the filtrate being shaken.

ii. Approximately 10 minutes later, the material with completely developed color of blue is subjected to spectrophotometry through a filter of 660 mμ.

e) Blank Determination for Anson's Method

Procedures are entirely identical until above mentioned process of b). Here trichlor acetic acid of 0.3 N is added to substrate solution. After well stirred, the mixture of b) is added and filtrated 30 minutes later. One ml of tyrosine solution prepared with hydrochloric acid of 0.1 N, which contains formalin as preservative in a proportion of 0.5 per cent, to be 0.0008 mEq/ml is added and the color is developed as in the above.

f) Activity of Control Trypsin Solution

In this determination, the material was replaced with distilled water, and the procedures otherwise were completely similar to the actual determination.

g) Standard Tyrosine Solution

Tyrosine is dissolved in 0.2 N hydrochloric acid, containing formalin for preservative in concentration of 0.5 per cent, to be 0.0008 mEq/5ml, which is similarly developed to produce adequate color.

h) Calculations

i. Tyrosine in digested filtrate $(\text{mEq}) = \frac{S}{D} \times 8 \times 10^{-4}$

ii. Tyrosine in the blank $(\text{mEq}) = \left(\frac{S}{B} \times 8 \times 10^{-4} \times \frac{19}{18} \right) - 8 \times 10^{-4}$

iii. Correction of the blank value = i.—ii.

S = Transparency of standard tyrosine solution

D = Transparency of digested filtrate

B = Transparency of the blank in e).

iv. In occasion of control trypsin solution, the calculations are entirely similar.

v. The value of tyrosine obtained from iii. and iv. are converted to trypsin activity using previously drawn standard curve.

vi. Trypsin activity of material filtrate is substracted from that of the control.

vii. Activity of trypsin inhibitor in the material of 1 ml is obtained from the values obtained from above mentioned calculations.

8. Determination of A. T. A.: GROB's method[19] was employed in A. T. A. determination, and trypsin activity was determined using hemoglobin substrate following ANSON's method[1)2)]. Results were represented in the term of A. U./ml. Essentials of the determination are summarized as follows.

i. The material is diluted 100 times with pH 7.6 phosphate buffer solution of N/10.

ii. Previously prepared and preserved trypsin solution is similarly diluted 50 times with the buffer solution.

iii. Diluted trypsin solution of 0.5 ml is added to 0.5 ml of diluted solution of the material, which is kept 15°C for 20 minutes.

iv. Trypsin activity is determined by Anson's method 20 minutes later.

v. As the blank determination, procedures are completely identical using buffer solution instead of diluted trypsin solution. By this determination, proteolytic activity in the material can be known, which is, however, as small a value practically, as well be neglected.

vi. Blank determination is performed in the same manner as in the determination of P. T. I.

vii. For the control, the determination is similarly carried out using the same amount of buffer solution instead of diluted material.

viii. Calculations are similar to those in P. T. I. determination. Here the proteolytic activity in the material should be substracted from the results as blank according to the following formulas.

〔Value of iv—Value of vi〕—〔Value of v—Value of vi〕 ……(1)

Value of vii—Value of vi ……(2)

Data of (1) and (2) are converted to trypsin activity by the use of the standard curve. From the difference of these two, antitryptic activity per ml of the original material can be sought.

9. Determination of A. T. A. in Tissues: The determination was done being based on the method of tissue antiplasmin determination of MACFARLANE and BIGGS[34)].

i. Approximately 1 g of piece of tissue taken is adequately rinsed with saline solution to remove blood.

ii. Piece of the tissue is dried being held between 2 sheets of filter paper.

iii. It is then weighed accurately to the extent of 10 mg.

iv. Appropriate amount of saline solution, generally 5 to 6 volumes, is added to the piece of the tissue, which is homogenized being cooled with water. Homogenous suspension thus obtained is centrifuged.

v. The supernatant is diluted with N/10 phosphate buffer solution of pH 7.6 finally to become 100 times dilution, including the dilution with saline.

vi. Activity of trypsin inhibitor of the diluted material is determined following the method of GROB.

vii. Antitryptic activity per 1 g of tissue thus obtained is represented in the term of A. U.

10. Crystalline Trypsin: "Tripure" (Novo Industry) was used for the control of trypsin inhibitor determination. Each vial contains 50 mg of crystalline trypsin (1.25 A. U.) with calcium salt as stabilizer[20]. Crystalline trypsin is solved in 0.0025 N hydrochloric acid in a concentration of 1 mg/ml finally being pH 3.0, which is preserved in refrigerator and newly prepared every two weeks. The trypsin solution is diluted as described in the above with the buffer solution to be pH 7.59 which is most suited for trypsin to act.

11. B. A. A. Substrate Solution: B. A. A. (Daiichi Chem. Indust.) was dissolved in N/15 phosphate buffer solution of pH 7.8 to a concentration of 0.1 M.

12. Indicative Reagent: In the determination following the method of NARDI, solution of 5 volumes of 0.1 per cent absolute alcohol solution of brom cresol green and 1 volume of 0.1 per cent absolute alcohol solution of methyl red was used. For adjusting pH in the method of KALSER-GROSSMAN, solution of brom thymol blue was used.

13. Hemoglobin Substrate[2]: Eight ml of 1 N NaOH, 36 g of urea, 10 ml of 22 per cent hemoglobin solution (Merck) and 72 ml of distilled water are mixed together and incubated at 25°C for 30 to 60 minutes to debase hemoglobin. After incubation, 10.0 ml of 1 M KH_2PO_4, 4 g of urea and approximately 2 g of Mersonin, as preservative are added finally to become 100 ml in all. The substrate solution is preserved in refrigerator and newly prepared every 2 weeks.

14. Microbiurette: Microbiurette (Shibata Chem. Co.) of precision type having total capacity of 0.1 ml with graduation of 1 microliter.

15. Phenol Reagent: Phenol reagent (Daiichi Chem. Indust.) following the recipe of FOLIN-CIOCALTEAU was used being diluted 3 times with distilled water.

16. Spectrophotometer: Spectrophotometer Junior Model 6 A, No. A 41453 (Coleman Co.) was used.

17. Measurement of Blood Pressure: A cannula was inserted into the femoral artery, which was connected to a mercurial manometer. As an anticoagulant, oxalate was used instead of commonly used heparin that has antitryptic property[22].

18. Standard Curve: In the process of spectrophotometry in ANSON's method, a standard curve should be drawn which shows the relationship between tranparency i. e. concentration of tyrosine and unit of trypsin activity. Amount of newly produced tyrosine at various concentrations of crystalline trypsin is plotted on a graph with tyrosine concentration (mEq) in vertical axis and trypsin activity (A. U.) in horizontal axis. Thus obtained curve is used as the standard.

III RESULTS

1. Changes in Active Trypsin and Trypsin Inhibitor caused by Laparotomy alone

In order to clarify the influence of laparotomy alone on the determination, trypsin activity, P. T. I. and A. T. A. in blood were determined before and after false laparotomy. Results obtained are shown in Fig. 1. It is considered to be quite natural that there should be no significant change in trypsin activity and P. T. I., since the procedure of the false laparotomy does not hurt the pancreas itself. However, in the change in A. T. A., two peaks were observed 6 hours and 72 hours postoperatively the latter being

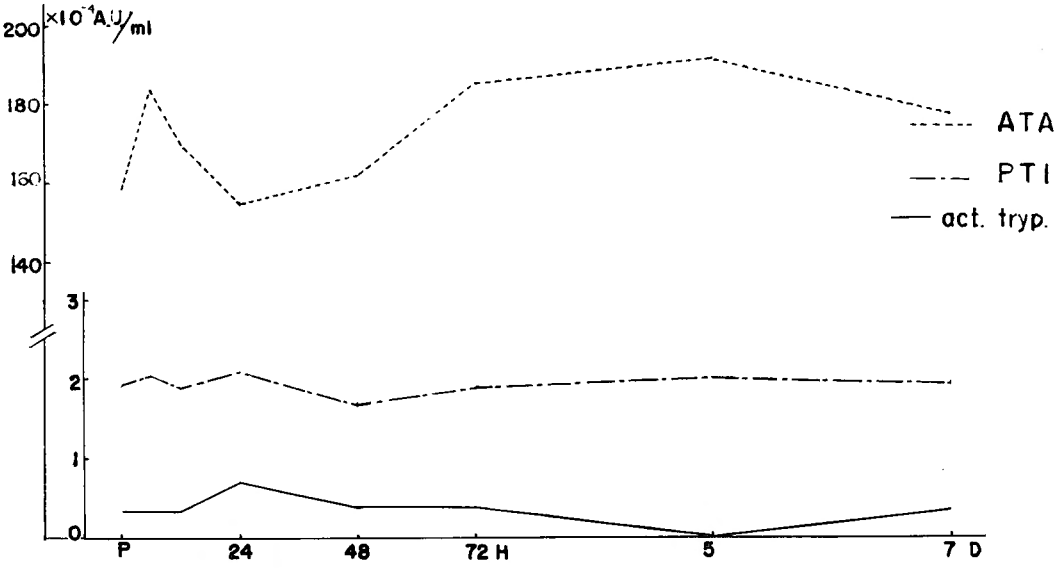

Fig. 1 Active trypsin & inhibitor in serum at exploratory laparotomy

persistent until 7 days after the operation.

Determinations of these properties were performed in dogs of acute pancreatitis. There was much difference in trypsin activity or activity of trypsin inhibitor between cases of early death with serious symptoms and those of long survival or complete recovery, even though acute pancreatitis was produced in the same manner. Accordingly, the animals were classified into 2 groups of early death within 48 hours and long survival.

2. Changes in Serum Trypsin and Trypsin Inhibitor at Acute Pancreatitis

Active trypsin, P. T. I. and A. T. A. in serum in dogs of acute pancreatitis are shown in Tab. 1 to 3, and the average values of these are summarized in Fig. 2.

a. Active Trypsin; Active trypsin can be found even in normal cases, but it showed, after onset of the disease, rapid then mild increase reaching its peak of 2.89×10^{-4} A. U. in cases of early death, and 2.68×10^{-4} A. U. in cases of long survival, on the average, 24 hour later, which was followed gradual decrease to restore to normal level or more low level towards 7th day of the disease, sometimes reaching zero.

b. P. T. I.; In normal cases, it ranged around 1.7×10^{-4} A. U. on the average. In cases of early death, it decreased slightly until 24th hour and in those of long survival slight fluctuation could be observed. In the latter, P. T. I. showed delayed increase compared with that in active trypsin, reaching its maximum value of 4.79×10^{-4}, on the average, 5 days after the onset of the disease, which is followed by gradual decrease thereafter to final level of normal, 14 days after the onset of the disease.

c. A. T. A.; Although individuality of A. T. A. in normal animals was larger than above mentioned two, the fluctuation showed similar tendency. Namely, it decreased markedly in cases of early death 6 hours after the onset of the disease, being followed by further decrease until 12th hour of the disease. Towards 24th hour of the disease, it showed an increase, but it remained below preoperative level, with only one exception. Obvious and

Table 1 Active trypsin in serum unit : 10^{-4} A. U./ml.

	Case of early death					case of long survival								
Dog. No.	Preop.	6H	12H	24H	Dog No.	Preop.	6H	12H	24H	48H	72H	5D	7D	10D	14D
S-1우	1.06	2.82	3.17		S-10우	0.71	2.12	2.82	2.82	2.47	1.06	1.06			
S-2♂	0.35	1.41	1.76	1.76	S-11우	0.71	1.41	2.82	3.17	2.47	1.06	1.41			
A-9우	0.71	1.76	1.76	2.12	A-16♂	0.35	1.41	1.76	2.12	1.76	1.76				
S-3우	1.06	2.47			S-12우	1.06	1.76	2.12	2.82	2.47	1.76	1.06	0.71	0.35	0.35
S-4우	0	1.76	2.12	2.82	A-15우	1.06	2.12	2.47	2.47	2.82	2.12	1.41	0.71	0.35	0
S-5♂	0.71	1.76	3.53	1.26	A-18우	0.71	1.06	1.76	2.12	2.12	1.06	0.71	0.35	0	0
A-7♂	0.71	2.12	2.47		A-17우	1.06	1.76	2.82	2.47	2.82	1.76	1.41	1.06		
A-1♂	1.06	2.82	3.17		A-19♂	0.88	2.33	2.88	2.50	2.25	1.05	0.70	—	0.35	
S-6♂	0.71	1.76	2.12	2.48	S-13우	0.71	2.12	2.47	3.17	2.82	2.12	0.71	0.35	0	0.35
A-3♂	0.88	1.05	2.08		S-14우	1.06	1.41	2.12	2.82	2.82	2.47	1.76	1.06	0.71	
A-4♂	1.25	1.45	2.30	2.05	A-23우	0	2.12	3.53	3.17	2.47	1.41	1.06	0.35		
S-7♂	1.41	2.47	2.82	3.53	S-15우	0.35	1.41	2.47	3.53	3.17	1.76	1.06	0.71	0	
A-6♂	0	2.12	2.62	3.17	S-16우	0.35	1.76	2.12	2.82	2.12	1.06	1.06	0.71	0.35	0.35
S-8우	1.06	1.41	2.82		S-17♂	0.71	1.76	2.12	3.53	2.47	1.41	0.71	0.35		
S-9♂	0.35	2.82	3.53	3.88	S-18♂	1.41	1.76	2.47	2.47	2.82	1.41				
					S-19우	0.71	1.41	2.47	2.82	2.82	1.41	0.71	0.71		
Mean	0.76	2.06	2.61	2.89	Mean	0.74	1.77	2.45	2.68	2.55	1.54	1.06	0.64	0.26	0.21

Table 2 P. T. I. in serum unit : 10^{-4} A. U./ml.

	case of early death					case of long survival									
Dog No.	Preop.	6H	12H	24H	Dog No.	Preop.	6H	12H	24H	48H	72H	5D	7D	10D	14D
S-1우	2.31	1.75	1.60		S-10우	1.98	1.72	1.87	2.20	2.49	3.02	4.04			
S-2♂	2.05	2.30	1.61	1.52	S-11우	1.90	2.29	1.85	1.70	2.41	3.80	5.18			
A-9우	2.47	2.40	1.76	1.76	A-16♂	2.35	—	2.13	2.54	3.09	4.31				
S-3우	0.68	1.82			S-12우	1.78	1.61	1.60	1.83	2.69	3.33	3.86	2.32	2.10	1.82
S-4우	1.56	1.58	1.22	1.03	A-15우	1.82	1.49	1.55	1.65	2.87	3.66	5.42	4.91	—	1.76
S-5♂	2.69	2.23	1.08	0.76	A-18우	1.58	1.69	1.81	1.90	2.51	3.35	4.84	4.06	—	2.37
A-7♂	1.53	1.39	1.25		A-17우	2.25	1.40	2.08	2.47	2.37	3.59	—	3.39	2.97	
A-1♂	0.88	0.94	0.72		A-19♂	2.00	2.31	2.39	2.62	3.34	3.81	4.53	—	3.70	
S-6우	2.06	1.48	1.66	1.21	S-13우	1.33	—	1.39	0.74	2.06	1.51	5.68	3.60	2.98	1.27
A-3♂	1.31	1.64	1.23		S-14우	1.19	—	0.87	1.54	2.58	4.30	5.03	4.86	3.51	
A-4♂	1.50	2.13	3.02	1.62	A-23우	1.06	1.10	0.92	0.86	1.23	3.21	4.61	1.69		
S-7♂	1.02	1.51	1.54	1.09	S-15우	1.98	1.45	1.51	1.40	2.15	3.75	6.00	4.96	3.09	
A-6♂	1.53	1.30	0.88	0.76	S-16우	1.42	1.70	2.78	2.66	3.47	3.32	3.93	2.68	—	2.05
S-8우	1.45	1.50	1.11		S-17♂	2.16	1.71	1.38	1.55	1.58	2.89	4.36	3.92		
S-9♂	1.27	1.25	1.13	1.16	S-18♂	1.52	1.35	1.57	1.96	2.48	3.29				
					S-19우	1.61	1.16	1.22	1.55	1.98	3.84	4.71	3.99		
Mean	1.69	1.68	1.41	1.21	Mean	1.75	1.61	1.67	1.82	2.52	3.62	4.79	3.67	3.38	1.85

Table 3 A. T. A. in serum unit : 10^{-4} A. U./ml

	case of early death					case of long survival								
Dog No.	Preop	6H	12H	24H	Dog No.	Preop	6H	12H	24H	48H	72H	5D	7D	10D	14D
S-1우	146	110	111		S-10우	154	124	146	198	229	197	184			
S-2♂	180	126	104	126	A-16♂	164	141	160	184	198	190				
A-9우	201	166	140	151	A-15우	137	98	145	170	211	209	196	179	160	158
S-3우	142	103			A-18우	189	157	193	165	174	151	140	145	126	113
S-4우	160	119	121	142	A-17우	222	159	208	206	217	202	210	208	185	
S-5♂	192	142	136	177	A-19♂	146	143	189	193	204	119	152	161	145	
A-7♂	189	145	145		S-13우	195	166	214	200	186	175	165	156	128	140
A-1♂	215	158	150		S-11우	181	132	186	161	155	—	141	143	140	
S-6우	179	154	139	160	A-23우	173	139	175	186	197	162	151	130		
A-3♂	197	125	128		S-15우	168	150	148	160	176	187	173	—	153	
A-4♂	186	150	134	141	S-16우	140	132	140	156	193	192	178	163	131	134
S-7♂	174	153	146	148	S-17♂	150	113	107	169	178	164	158	152		
A-6우	158	112	194	121	S-18♂	138	140	206	206	210	163				
S-8우	190	181	143		S-19우	205	172	201	182	205	173	167	154		
S-9♂	131	97	96	132											
Mean	176.0	136.1	128.4	141.2	Mean	168.7	140.4	177.8	181.2	195.2	177.9	167.9	159.1	143.5	136.3

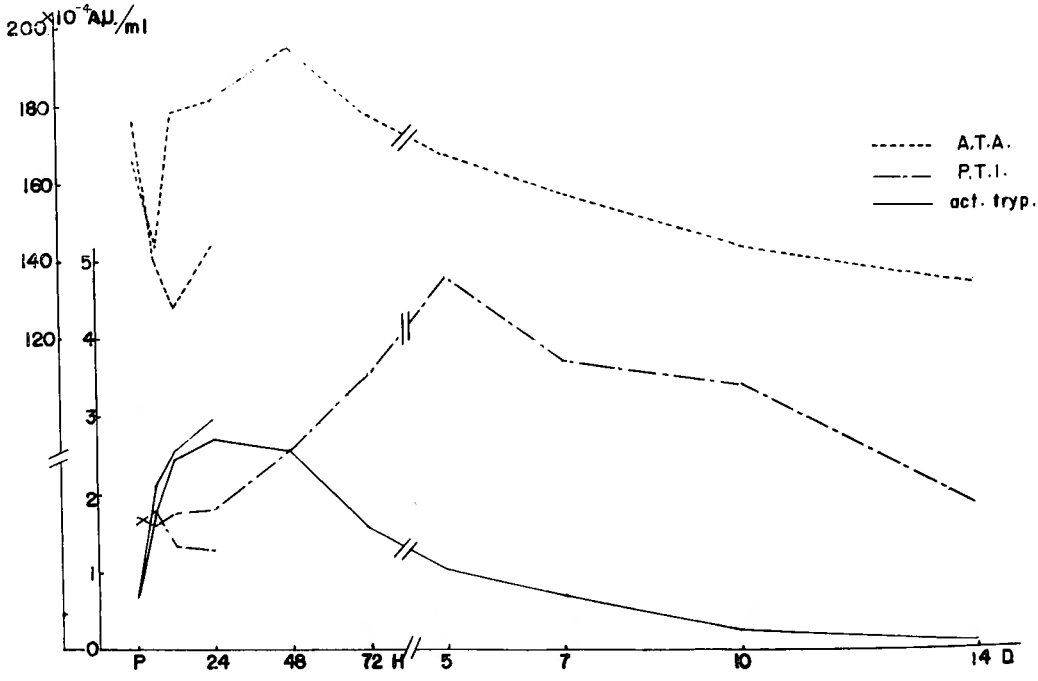

Fig. 2 Active trypsin, P. T. I. & A. T. A. in serum at acute pancreatitis (Mean value)

significant difference could be observed between cases of long survival and early death, in the former it decreasing rapidly at 6th hour and contrariwise increasing to exceed the preoperative level at 12th hour of the disease, in most cases. The increase still continued on thereafter to 195.2×10^{-4} A. U., 48 hours after the onset of the disease, on the average, which was followed by gradual decrease to restore to normal level towards 5th day of the disease, showing further decrease in most cases.

3. Changes in Active Trypsin and Trypsin Inhibitor in Peritoneal Fluid at Acute Pancreatitis

Relationship between active trypsin and trypsin inhibitor was studied with the materials of peritoneal fluid and serum simultaneously taken, data of which are shown in Tab. 4 to 6, each average value in Fig. 3 to 5. As the amount of peritoneal fluid lessened over 72 hours after the onset of the disease practically, and it became hard to collect the fluid owing to progressive intraperitoneal adhesion or the fluid became supprative at this

Table 4 Active trypsin in serum and peritoneal fluid unit : 10^{-4} A. U./ml
S : serum, A : peritoneal fluid
upper : case of early death lower : case of long survival

Dog No.	Preop. (S)	6 H		12 H		24 H		48 H		72 H	
		S	A	S	A	S	A	S	A	S	A
A-1 ♂	1.06	2.82	3.17	3.17	3.17						
A-2 ♀	0.71	2.12	2.12	2.12	2.47	2.47	2.47				
A-3 ♂	0.88	1.05	1.88	2.08	2.13						
A-4 ♂	1.25	1.45	2.85	2.30	1.75	2.05	2.13				
A-5 ♀	1.05	2.47	2.82	2.82	3.53	3.17	2.82				
A-6 ♂	0	2.12	2.47	2.47	3.17	3.17	3.17				
A-7 ♂	0.71	2.12	2.47	2.47	2.82						
A-8 ♀	0.35	0.35	2.47	3.17	4.26	3.53	3.17				
A-9 ♀	0	1.76	2.12	1.76	2.47	2.12	2.82				
A-10 ♂	0.71	1.76	2.47	2.12	2.82	2.82	2.47				
A-11 ♀	1.41	2.82	3.53	2.82	2.82	3.17	2.82				
A-12 ♀	0.35	2.12	2.82	2.12	3.53	2.45	—				
Mean	0.70	2.03	2.56	2.61	2.89	2.75	2.73				
A-13 ♂	0.71	1.41	2.12	2.18	2.47	2.82	2.82	2.47	2.82	1.76	1.76
A-14 ♀	0.71	1.06	2.82	2.82	3.17	3.53	3.53	2.82	3.17	1.41	2.12
A-15 ♀	1.06	2.12	1.76	2.47	2.12	2.82	2.82	2.82	2.47	2.12	2.12
A-16 ♂	0.35	1.11	1.76	1.76	2.12	2.12	2.47	1.86	2.12	1.76	1.76
A-17 ♀	1.06	1.76	2.47	2.82	2.82	2.47	2.12	2.82	2.47	1.41	1.41
A-18 ♀	0.71	1.06	1.41	1.76	1.76	2.12	1.76	2.47	2.12	1.41	1.76
A-19 ♂	0.88	2.33	2.45	2.88	2.30	2.50	2.63	2.25	2.08	1.05	1.25
A-20 ♂	0	1.41	2.12	2.12	2.82	2.82	2.47	2.47	2.82	2.12	2.12
A-21 ♀	0.35	1.76	2.82	2.47	2.82	2.82	3.17	2.47	2.82	2.12	2.12
A-22 ♂	0.71	1.76	2.82	2.47	2.82	3.53	3.17	3.17	3.17	1.06	2.12
A-23 ♀	0	2.12	2.82	3.53	2.47	3.17	2.82	2.82	2.82	1.41	1.76
Mean	0.59	1.64	2.26	2.16	2.50	2.77	2.69	2.56	2.63	1.53	1.81

Table 5 P. T. I. in serum and peritoneal fluid unit : 10^{-4} A. U./ml
S : serum, A : peritoneal fluid
upper : case of early death lower : case of long survival

Dog No.	Preop. (S)	6 H		12 H		24 H		48 H		72 H	
		S	A	S	A	S	A	S	A	S	A
A-1 ♂	0.88	0.94	1.05	0.72	1.05						
A-2 ♀	1.62	1.47	1.41	1.36	1.56	1.24	1.90				
A-3 ♂	1.34	1.64	1.59	1.23	1.41						
A-4 ♂	1.50	2.13	1.73	3.02	1.92	1.62	2.26				
A-5 ♀	1.78	1.56	1.20	1.49	1.33	1.15	2.04				
A-6 ♂	1.53	1.30	0.88	0.88	0.80	0.76	1.57				
A-7 ♂	1.53	1.39	1.45	1.25	1.18						
A-8 ♀	2.21	2.08	0.96	1.11	1.07	0.83	2.02				
A-9 ♀	2.47	2.40	0.81	1.76	1.12	1.76	1.33				
A-10 ♂	1.99	1.80	1.13	1.57	1.38	1.42	1.95				
A-11 ♀	1.67	1.31	1.07	1.17	1.29	1.21	2.15				
A-12 ♀	2.15	1.71	1.33	1.68	1.16	1.44	1.54				
Mean	1.72	1.64	1.22	1.44	1.27	1.27	1.83				
A-13 ♂	2.28	2.12	1.40	1.58	1.48	1.71	1.45	2.31	1.56	2.17	1.73
A-14 ♀	1.55	1.58	1.02	1.16	1.54	1.89	2.27	2.43	2.20	3.85	2.01
A-15 ♀	1.82	1.49	1.32	1.55	1.61	1.65	1.78	2.87	1.65	3.66	1.90
A-16 ♂	2.35	—	1.50	2.13	1.94	2.54	2.16	3.09	2.08	4.31	—
A-17 ♀	2.25	1.40	1.16	2.08	1.35	2.47	1.84	2.37	1.12	3.59	1.38
A-18 ♀	1.58	1.69	0.86	1.81	1.58	1.90	2.59	2.51	2.11	3.35	2.57
A-19 ♂	2.00	2.31	2.02	2.38	2.09	2.62	2.21	3.34	1.99	3.81	1.75
A-20 ♂	1.22	1.56	1.31	1.44	2.13	1.78	2.06	2.70	2.43	3.89	2.16
A-21 ♀	1.40	1.45	—	1.51	1.71	1.40	2.49	2.15	1.88	3.75	2.33
A-22 ♂	1.74	1.65	1.84	1.85	2.29	2.13	1.98	2.61	1.66	3.26	1.34
A-23 ♀	1.05	1.10	1.63	0.92	1.80	0.86	1.73	1.23	1.84	2.24	2.62
Mean	1.76	1.61	1.54	1.70	1.77	1.88	2.10	2.51	1.89	3.56	1.98

stage, making the data incredible, collection of peritoneal fluid was carried out until 72nd hour of the disease even in cases of survival.

a. Active Trypsin ; In both groups of early death and long survival, active trypsin of peritoneal fluid slightly exceeded that of serum. The difference gradually became smaller, becoming obscure in general 24 to 48 hours after the onset of the disease. Active trypsin of peritoneal fluid, however, exceeded, though slightly, that of serum 72 hours after the onset of the disease.

b. P. T. I. ; P. T. I. in peritoneal fluid was generally less than in serum 6 hours after the onset of the disease, which increased on gradually in both groups of early death and long survival in most cases, exceeding the level in serum 24 hours after the onset of the disease. It is noticeable that P. T. I. level of peritoneal fluid was persistent to the level of about 2×10^{-4} A. U., while P. T. I. of serum increased thereafter.

c. A. T. A. ; A. T. A. of peritoneal fluid and serum showed resembling curves to

Table 6 A. T. A. in serum and peritoneal fluid

Dog No.	Preop. (S)	6 H S	6 H A	12 H S	12 H A	24 H S	24 H A
A-1 ♂	186	170	107	151	110	166	108
A-5 ♀	161	150	122	145	118	138	120
A-6 ♂	158	122	91	111	89	151	106
A-2 ♀	151	143	110	148	109	160	119
A-7 ♂	189	155	94	155	96		
A-24 ♂	202	161	126	138	107	149	118
A-3 ♂	197	145	99	148	106		
Mean	177.7	149.4	107.0	142.7	105.0	152.8	111.2

S : serum
A : peritoneal fluid
unit : 10^{-4}A.U./ml
upper : case of early death
lower : case of long survival

Dog No.	Preop. (S)	6 H S	6 H A	12 H S	12 H A	24 H S	24 H A	48 H S	48 H A	72 H S	72 H A
A-17 ♀	165	149	102	167	125	166	130	163	124	134	118
A-23 ♀	222	189	129	218	151	206	159	227	154	202	145
A-19 ♀	173	149	105	175	120	186	136	197	138	162	111
A-21 ♀	146	153	96	189	141	193	140	224	150	149	108
A-18 ♀	157	140	127	162	118	175	154	203	166	156	133
A-20 ♂	189	157	113	203	136	165	110	174	108	151	95
Mean	175.3	156.2	112.0	185.7	136.8	181.8	138.8	198.8	140.0	159.0	118.3

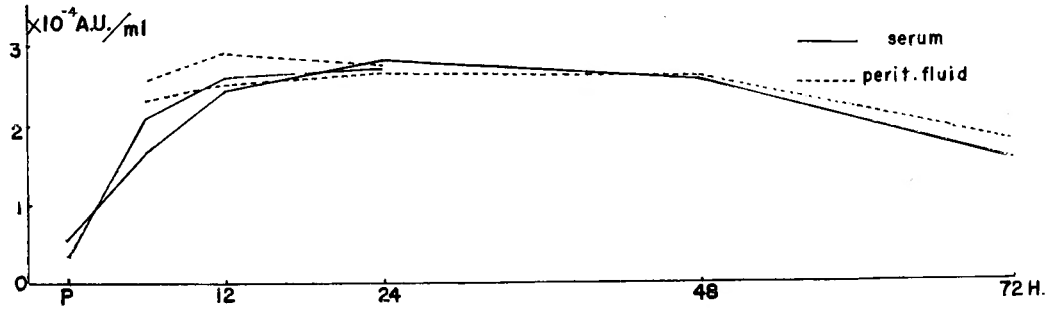

Fig. 3 Active trypsin in serum & peritoneal fluid (Mean value)

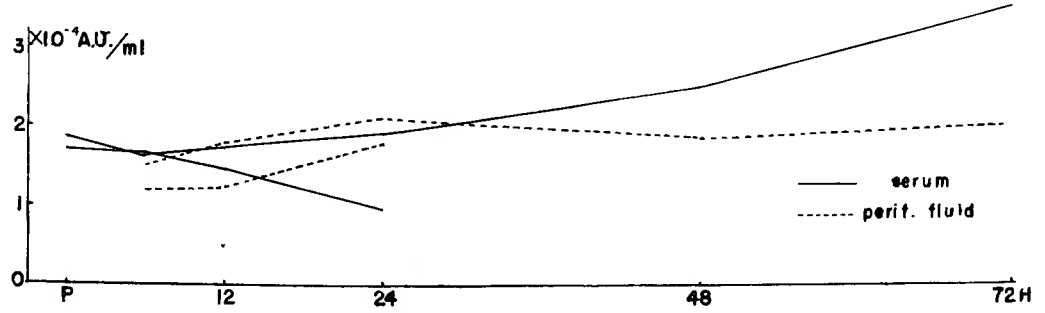

Fig. 4 P. T. I. in serum & peritoneal fluid (Mean value)

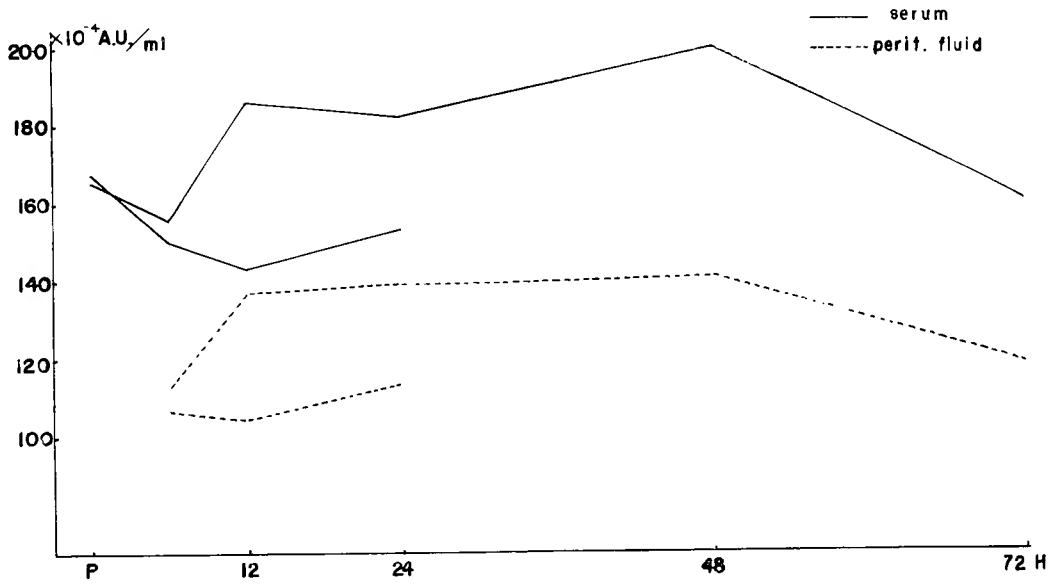

Fig. 5 A. T. A. in serum & peritoneal fluid (Mean value)

each other, the former never exceeding the latter and being kept approximately on the level of 75 per cent of the former. The difference between cases of early death and those of long survival was also most prominent in A. T. A. as in serum.

4. Changes in Active Trypsin, Trypsin Inhibitor and Blood Pressure at Intravenous Injection of Crystalline Trypsin in Dogs survived Acute Pancreatitis

In order to study the response against injection of trypsin of considerably large amount in dogs survived acute pancreatitis (abbreviated to "survived dog", hereafter), fluctuation of blood pressure, active trypsin in blood and inhibitor expressed as A. T. A., in the survived dogs in which trypsin solution was injected from the femoral vein spending 10 minutes, were pursued. Trypsin solution was prepared in 25 ml of saline in a proportion of 4 mg per kg body weight. Results obtained are summarized in Fig. 6 and 7.

a. Active Trypsin; Although it is quite natural that active trypsin should show the maximum of 5.56×10^{-4} A. U. immediately after the injection in normal animals, it was followed by relatively rapid decrease until 60 minutes after the injection reaching a half level compared with the maximum. However, decrease thereafter was mild, showing a level as 1.45×10^{-4} A. U. even 150 minutes after the injection. On the other hand, the maximum of survived dogs was a little lower than in normal ones, being 5.1×10^{-4} A. U., which was followed by a certain decrement thereafter, restoring to normal as early as 100 minutes after the injection, and trypsin activity could not be observed 120 minutes after it.

b. A. T. A.; In normal dogs, A. T. A. slightly increased as shown in the figure, with following decrease, while in survived dogs it decreased from the beginning. A.T.A. in both animals decreased revealing similar level thereafter until 60 minutes after the injection. Later than 60 minutes, A. T. A. in survived dogs showed a tendency of gradual increase with slight fluctuation, whereas in normal dogs it continued to decrease on finally

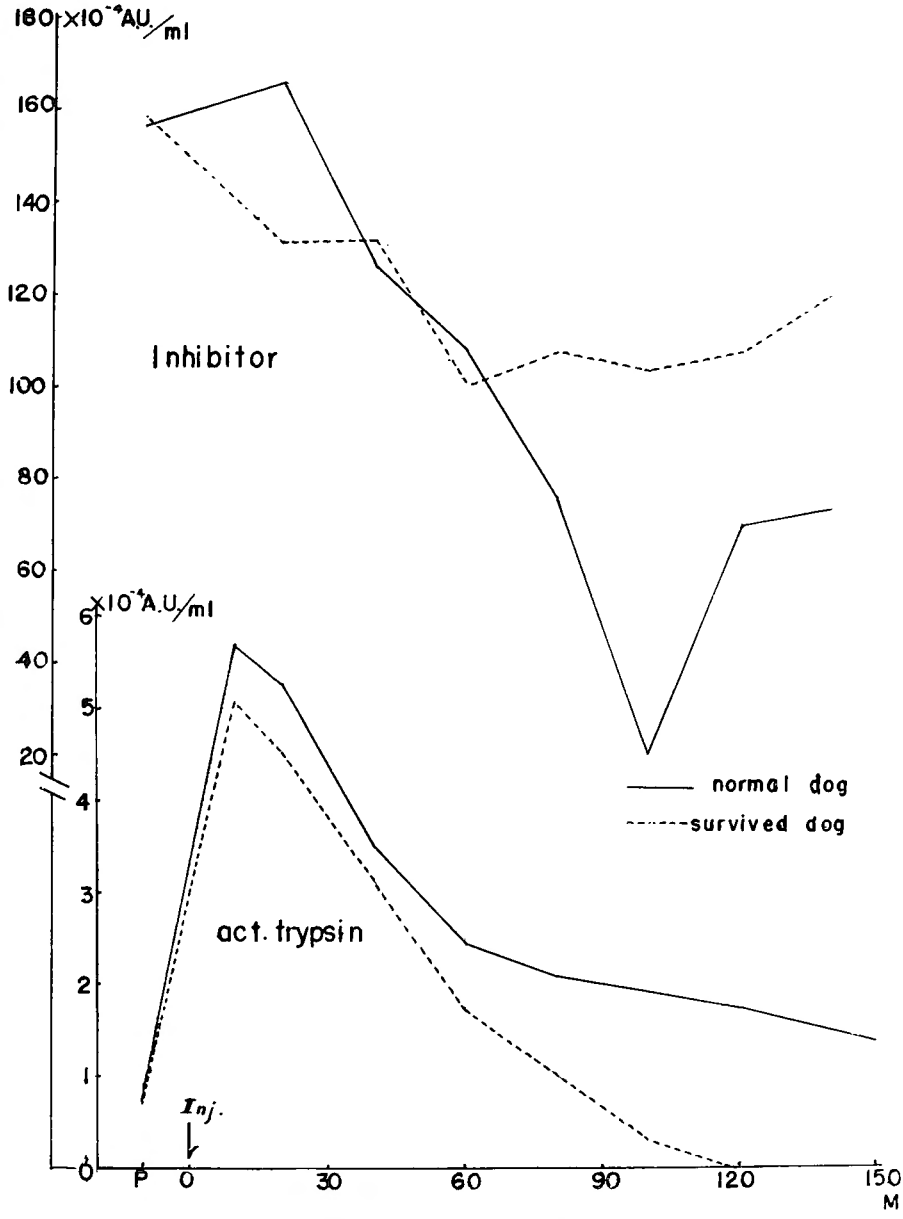

Fig. 6 Inhibitor & active trypsin after intravenous injection of crystalline trypsin

to reach as low a level as 20×10^{-4} A. U. 100 minutes after the injection and it tended to restore as late as 120 minutes later.

c. Blood Pressure ; Animals of both experiment and control showed the symptoms of shock with marked descension of blood pressure 5 minutes after the injection, when the injection was not finished yet. Survived dogs, however, soon recovered showing blood pressure of about 70 mmHg, 20 to 100 minutes after the injection, and further, showing the restoration to normal level later than 130 minutes, while in normal dogs blood pressure

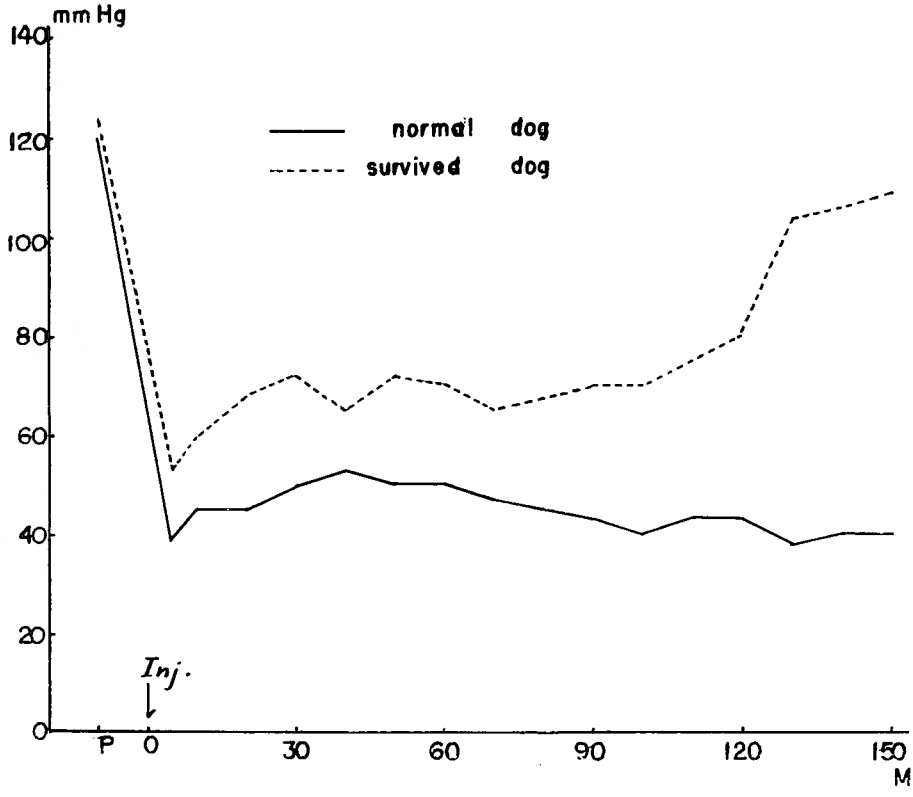

Fig. 7 Blood pressure after intravenous injection of crystalline trypsin

remained within the range of 40 to 50 mmHg until 150 minutes after the injection, showing no sign of recovery.

In this experiment, 25 ml of saline was injected in the similar way for the control. In these animals, blood pressure and A. T. A. were examined with the outcome of temporary and slight fluctuation of blood pressure alone.

5. Changes in Antitryptic Activity in Various Organs at Acute Pancreatitis and after Intravenous Injection of Crystalline Trypsin

As in the previous experiment, trypsin was injected intravenously and specimens were taken from the liver and spleen before and after the injection, and A. T. A in the homogenate of these pieces of the organs was determined. Results obtained are shown in Fig. 8 together with active trypsin and A. T. A. in blood simultaneously determined. Organ specimens were taken only 3 times, i. e. before the injection, 60 minutes and 120 minutes after the injection, which was so confined being related to the time of biopsy. A. T. A. in the liver of normal dogs decreased and that in the spleen showed hardly discernible change, whereas in survived animals A. T. A. in both of the liver and spleen increased, though slightly, with obvious difference from those of normal animals. Here, trypsin inhibitor of the liver and spleen were determined as well as A. T. A. in blood in experimentally produced acute pancreatitis. The results are shown in Tab. 7 and the average values are summarized in Fig. 9. Compared with activity of trypsin inhibitor in 1 ml of

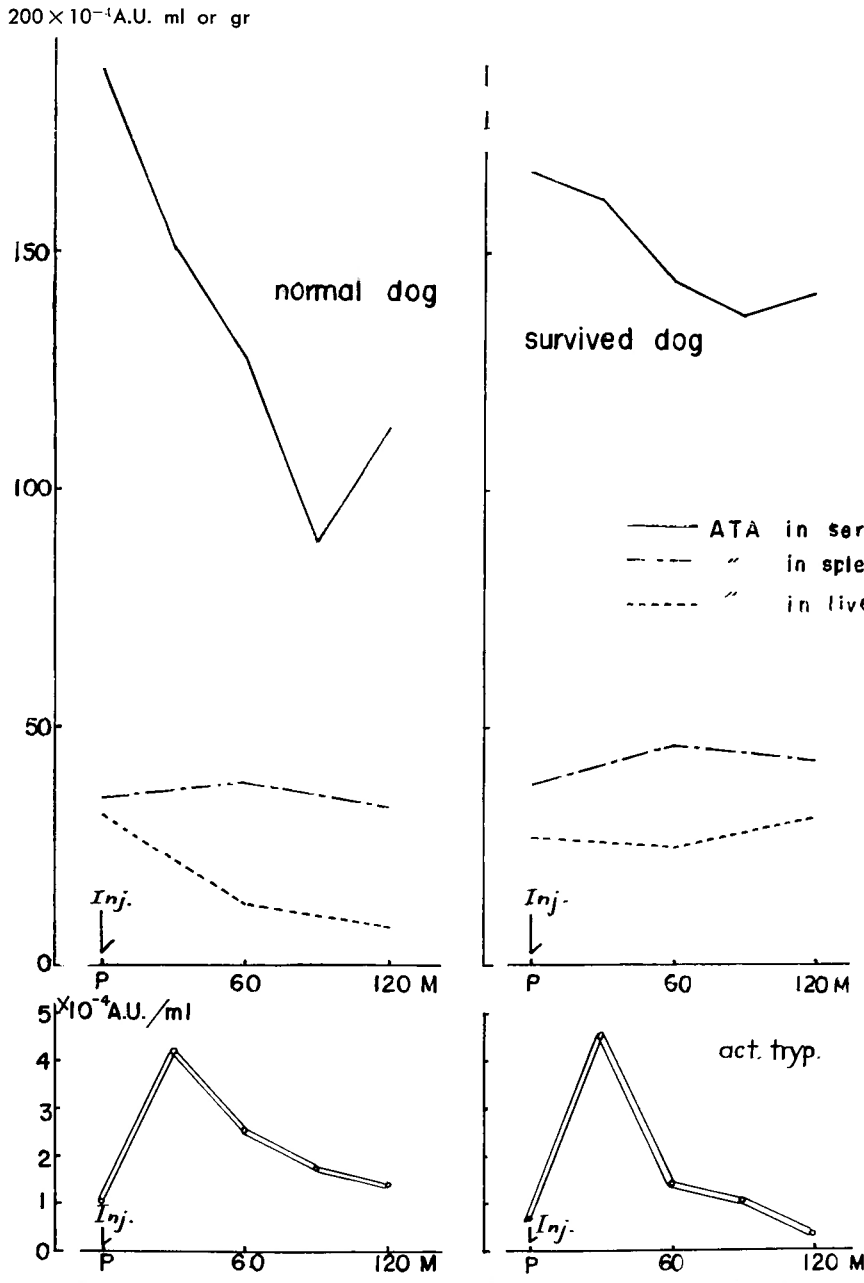

Fig. 8 Active trypsin & inhibitor in serum & tissues after intravenous injection of crystalline trypsin

serum, that of trypsin inhibitor per 1 g of tissue could be said to be low. Nevertheless, what was so interesting was that changes in tissue A. T. A. and serum A. T. A. was inversely proportionate to each other until 24 hours after the onset of the disease, and particularly that A. T. A in the liver increased rapidly and temporarily showing steep peak of as high a level as twice of the preoperative level 6th hour of the disease, as if respond-

Table 7 A. T. A. in serum and tissues
left : case of early death right : case of long survival

Dog No.		Preop.	6H	12H	24H	Dog No.		Preop.	6H	12H	24H	48H	72H
	S	196	166	149	165		S	184	133	186	197	201	168
T-1 우	L	47.3	46.4	51.4	31.5	T-6 ♂	L	34.7	81.4	35.4	30.6	51.3	73.9
	M	53.0	70.8	72.2	36.3		M	35.0	56.9	40.8	42.2	58.5	62.4
	S	181	156	153	177		S	196	135	155	203	211	172
T-2 ♂	L	31.1	60.0	57.2	56.1	T-7 우	L	26.8	104.4	60.3	35.2	52.4	58.0
	M	56.8	55.5	51.9	46.8		M	41.4	48.5	54.3	43.4	42.6	50.7
	S	166	107	113	118		S	154	118	148	180	187	158
T-3 우	L	34.8	72.6	46.1	41.5	T-8 ♂	L	41.1	83.8	67.9	51.3	63.1	71.7
	M	36.9	37.0	41.8	31.4		M	50.6	65.4	58.5	55.0	55.6	62.5
	S	183	127	115	154		S	138	106	219	208	213	147
T-4 ♂	L	56.7	70.3	71.2	47.0	T-9 ♂	L	56.0	95.9	71.9	68.2	79.1	82.9
	M	51.5	57.4	67.2	60.6		M	64.1	68.3	73.7	64.6	—	79.9
	S	159	136	121			S	171	148	163	156	205	176
T-5 우	L	35.8	44.3	48.0		T-10 우	L	36.2	53.6	52.0	43.1	83.3	96.8
	M	43.9	50.2	52.0			M	40.2	49.9	57.5	56.1	70.8	71.4
	S	177.0	134.4	132.7	153.5		S	168.6	128.0	171.2	188.8	203.4	164.2
Mean	L	41.1	58.7	56.0	44.0	Mean	L	39.0	83.8	57.5	45.7	66.4	76.7
	M	48.4	51.2	57.0	43.8		M	46.3	57.8	57.0	52.3	56.9	65.4

S : serum L : liver M : spleen unit : 10^{-4} A. U./ml. or gm.

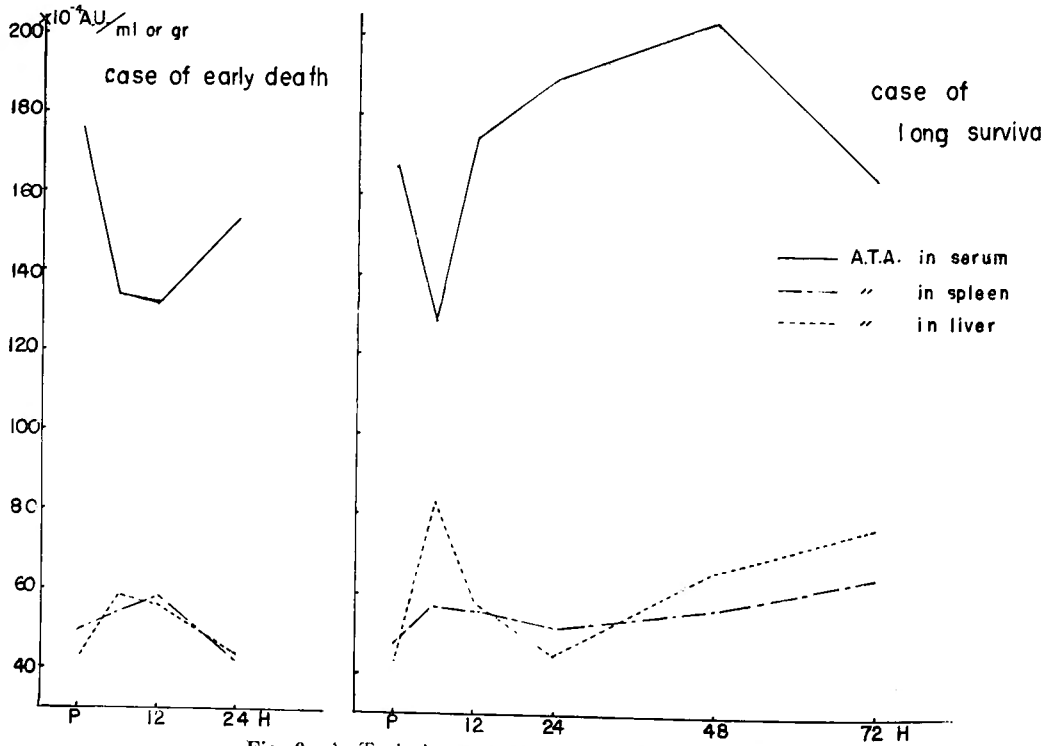

Fig. 9 A. T. A. in serum & tissues at acute pancreatitis

ing to temporary rapid decrease in A. T. A. in serum. Thus, A. T. A. in the tissues continued to increase gradually later than 24 hours after the onset of the disease regardless of the change in serum A. T. A., A. T. A. of the liver constantly showing higher level than that of the spleen. In general, changes in A. T. A. of the liver was more marked than that in A. T. A. of the spleen.

IV. DISCUSSION

There have been many reports on the method of producing experimental acute pancreatitis, such as ligation of the pancreatic duct, ligation of the pancreatic vessels, injection of olive-oil, bile of pig, trypsin or intestinal content into the pancreatic duct and combinations of these. Among these, injection of autogenous bile is the most simple and suited to the aim of producing clinical pictures as typical edema, hemorrhage or necrosis. Amount of bile for the injection was decided to be 0.3 ml per kg body weight, since too much serious pancreatitis was produced to pursue the relationship between trypsin and trypsin inhibitor, which was the original aim of the present experiment, when the bile of more than 0.4 ml per body weight was injected, and, on the contrary, when the bile was less than 0.2 ml per kg body weight, the injection resulted merely in the slightest edema, without producing pancreatitis of appropriate degree. Concerning the pressure for the injection, condition was maintained invariably by using the same syringe and needle (gauge 22) and injecting the bile spending 1 second for 0.5 ml, since too slow injection produced too slight picture of the disease. Success of the injection could be discerned from the change in the color of the pancreas into yellow-brown, several minutes afterwards.

There are many devices for the determination of proteolytic enzymes using various substrates of protein or synthetics[5)28)9)49)10)51)45)24)15)14)21)]. At present, no method can be found in literatures which enable precise determination of trypsin alone, among these enzymes having resembling physiochemical and enzymological character. On the other hand, although it is readily presumed that trypsin plays the principal role clinically as a proteolytic enzymes in acute pancreatitis, it is assumed that, besides trypsin, pancreatogenic chymotrypsin, carboxypeptidase, enzymes of kallikrein group and plasmin, as considered to be activated secondarily, interfer the enzymodynamics more or less, bringing forth a complicated picture. It was studied by BERGMANN[3)] as early as in 1939, that synthetic substrate B. A. A. (benzoyl-l-arginine amide hydrochloride) is decomposed almost exclusively by trypsin. NARDI's method[37)] reported in 1958 in which above mentioned characteristics of B. A. A. and CONWAY's[8)] microdiffusion analysis method are utilized, might well be considered to be a satisfactory method for the determination of trypsin activity, since the influence of other coexisting enzymes is so slight as to be neglected compared with trypsin itself, even though it cannot be said precisely that there is no interference of other enzymes in this method. Of course, there are some disadvantages in this method, the greatest problem is the limit of macroscopic reading of the terminal point of titration. Owing to macroscopic reading of the point of the change in color at titration, standard deviation is necessarily apt to expand. Accordingly, NARDI himself reported that standard deviation was ± 50 units, when the maximum normal value was determined to be 100 units. With the microbiurette as used in the present experiment, precision does not exceed the limit of 0.5 microliter i.

e. 0.35×10^{-4} A. U. Further development in the precision can be expected in the near future, since there are several reports[14)5)] to represent the results photometrically using B. A. A. substrate, although it was not executed in the present experiment.

In general, activity of trypsin inhibitor is represented by the activity of trypsin that was inhibited by trypsin inhibitor, and the amount of trypsin inhibited is proportionate to that of trypsin inhibitor[47)]. Accordingly, the principle of the determination consists in substracting residual trypsin activity from the original one after these two are let react to each other. There are various methods for the determination of trypsin inhibitor, i. e. antitryptic activity[28)10)49)13)17)11)45)24)]. There is, however, little difference in the principle of these methods using different substrate, buffer system or the judgement of the results of titration. It is a matter of course that the method employed here, i. e. GROB's method cannot be any exception and trypsin inhibitor of combined form is not determined. On the contrary, results of KALSER-GROSSMAN's method includes trypsin inhibitor of combined form besides that of isolated form, as the compound is decomposed during the process of extraction and trypsin forming the compound simultaneously precipitates leaving trypsin inhibitor within the solvent. Moreover, it is considered that the determination has better reproducibility when hemoglobin is used as substrate than other ones[1)].

In this method of trypsin inhibitor determination, the material is relatively well diluted, the principal aim of which, of course, consists in making the material appropriate concentration for photometry. However, more important significance therein should consists in weakening the activity of other proteolytic enzymes by dilution in order to avoid untoward interference of these enzymes by lessening these compared with trypsin added afterwards. With the only exception of tissue extract, value of blank determination devoid of trypsin was so small as to be neglected. In addition, it is said that there is interfering effect against the reaction in low dilution of serum[47)]. According to the report of JACOBSSON[24)], antitryptic inhibition of serum is directly proportionate to the amount of serum added up to 70 per cent inhibition. In the present experiment, it was ascertained by examining above mentioned dilution that inhibition was below 70 per cent.

Although some literatures could be seen, which admit or presume existence of trypsin inhibitor in the tissue[5)], no literature could be found concerning the method of the determination. Of course, it is not clear whether or not trypsin inhibitor within the tissue be identical to the substance already known such as trypsin inhibitor in serum. MACFARLANE[34)] reported the results of determination of plasmin inhibitor in various tissues. According to the recently acquired knowledge, some of trypsin inhibitors so closely resemble plasmin inhibitor in its character that the determination of trypsin inhibitor in the tissues was carried out based on the method of MACFARLANE. It is known that plasmin inhibitor widely exists in the tissues of the entire body, and it is presumed that trypsin inhibitor also may possibly exist similarly. Accordingly, trypsin inhibitor should have been more widely pursued in various tissues at acute pancreatitis, but it was hardly possible in such experiment as to take tissue specimens of small amount frequently during the course of acute pancreatitis. Trypsin inhibitor of the tissue was determined in the liver and spleen in the present experiment based on the following reasons. According to MACFARLANE, the spleen which was taken in the present experiment is the organ that is the richest in plasmin. Further-

more, the liver is thought anatomically to be the first organ that possibly meets large amount of liberated trypsin at acute pancreatitis, and these two are suited to repeating biopsy because of their large volume.

It has been described and observed by many including NITTA in our clinic that various enzymes are liberated continuously to the surrounding tissues and peritoneal cavity and hemato- or lymphogenously to circulating blood at acute pancreatitis following destruction of the pancreatic tissue due to elevation of inner pressure of the pancreas and or autodigestion of the organ. Among these liberated enzymes, proteolytic ones such as trypsin are thought to be inactivated shortly in most part by corresponding inhibitory properties, and remarkable increase cannot be observed even by various methods of determination.

In the present experiment, first of all rapid increase in active trypsin in blood could be observed shortly after the production of acute pancreatitis. This finding is interpreted that trypsin itself, which is activated, even if a small amount, by some moment, acts as an activator of trypsinogen, and the rapid increase in trypsin might be the result of successive trypsin activation within the pancreas[51]. At this point, P. T. I. firstly acts to neutralize the increased trypsin, but as trypsin is once liberated from the pancreas, particularly into the blood stream, it must be inactivated by trypsin inhibitor most part of which may presumably be serum trypsin inhibitor and represented as A. T. A. in blood, richly existing there and having a large reserve capacity. Here, active trypsin and A. T. A. behaved during the entire course of the observation, as if in an enzymodynamic equilibrium, as shown in Fig. 2. At 6th hour of the disease, however, temporary decrease in A. T. A. could be observed as if replying to rapid increase in active trypsin observed at this stage. It is quite natural that this finding should be accepted to be due to neutralization of rapidly increased trypsin; and, on the other hand, it is also presumed to be due to transfer of serum trypsin inhibitor into the tissues. Rapid increase following this transient decrease might be attributed to the supply of trypsin inhibitor by some mechanism. This interpretation is particularly interesting when considered with the fact that early death occurred in a state of decrease in A. T. A., showing no tendency of recovery in A. T. A.

On the other side, P. T. I. showed obviously different change from that of trypsin with the maximum value at the 5th day of the disease, which well resembled the results of the change in antiproteolytic activity in the pancreatic tissue reported by ODA[56][61] It is assumed so long as Fig. 2 is concerned that there is no correlation between the changes in P. T. I. and other two properities of active trypsin and A. T. A. PECK and DENVER[42] maintained that most part of trypsin which entered blood stream is inactivated within relatively short period, which was also ascertained in the present experiment by injecting trypsin intravenously. This finding can be presumed to suggest that the amount of liberated trypsin at acute pancreatitis would be unexpectedly large, and on the contrary, that of P. T. I. participated in this process unexpectedly small. As already mentioned above, slightly higher level of active trypsin and markedly lower level of A. T. A. were observed in cases of early death compared with those in cases of long survival, it is considered to be significant as one of factors of development of the disease that together with the increase in liberated trypsin A. T. A. in blood or reserve supply of A. T. A. goes to decay.

Concerning the mechanism of peritoneal fluid accumulation, two different processes can be considered such as immediate exudation of fluid containing high concentration of enzymes through the pancreatic capsule, as was demonstrated by NITTA in our clinic, and exudation of relatively large amount of fluid from widely covering peritoneum. Although there is little difference between active trypsin in peritoneal fluid and serum, the former invariably exceeded the latter, which is particularly marked at initial stage within 12 hours after the onset of the disease. It is presumably due to the fact that at this stage exudate from the peritoneum does not come to sufficiently large amount compared with the amount of pancreatic exudate of high concentration. In other words, highly concentrated pancreatic exudate is not diluted and neutralized sufficiently at this stage. This interpretation is supported by the observation that P. T. I. in peritoneal fluid surpassed P. T. I. in serum. According to the finding of exploratory laparotomy, peritoneal fluid was most accumulated around 48th hour of the disease and later than 72th hour it decreased gradually, making its collection difficult. It is assumed that such dilution-effect works to maintain P. T. I. of peritoneal fluid in a constant level despite an increase in P. T. I. in serum later than 24 hours after the onset of the disease. On the other hand, A. T. A. in serum and peritoneal fluid changed in parallel, the latter being constantly in a level of about 75 per cent of the former, which is accepted to support the presumption that A. T. A. mostly originated from the peritoneal exudate than from pancreatic exudate.

Since the early experiments of GOODPASTURE and WHIPPLE[52], there have not been so many reports on the problem of peritoneal fluid accumulated at acute pancreatitis. There is an impression that toxicity of the fluid has been solely emphasized as a common conception. As is understood from the results of the present experiment, however, there still remains some problems to be studied concerning the significance of the peritoneal fluid. The peritoneal fluid is consisted of the fluid immediately originated from the pancreas and another one. It can be infered that the former, containing highly concentrated enzymes, acts more or less to stimulate the peritoneum, and the latter is produced reactively. It was clarified by NITTA that pancreatic exudate of small amount manifests a pronounced toxicity, which is weakened a great deal by peritoneal exudate produced a little later, the former being neutralized and diluted. For instance, trypsin activity is weakened nearly to the level of peripheral blood, and thus weakened fluid remains in the peritoneal cavity for certain period of time until absorbed gradually. Accordingly, it is naturally presumed that the toxicity of the peritoneal fluid against important organs and tissues is already weakened markedly compared with initial one.

It has been known that symptoms of intravenous injection of trypsin resemble so closely to those of acute pancreatitis clinically seen, in many respects. Accordingly, for the aim of studying pathophysiology of acute pancreatitis, it is meaningful to clarify behavior of trypsin inhibitor at trypsin injection to analogize the occasion of acute pancreatitis. As PECK and DENVER[42] reported, most of trypsin injected in the present experiment was inactivated within an extremely short period. Though it is a matter of course that active trypsin in blood showed the maximum immediately after the injection, it decreased on thereafter, particularly until 60 minutes later, almost entire trypsin injected being inactivated 120 minutes after the injection. This finding coincides with the result of ARAKI[54]. There

remains some problems to consider that such phenomenon is entirely due to trypsin inhibitor, however, in this process it is assumed that trypsin inhibitor has at least significance to considerable extent, and a rapid decrease in A. T. A. at this stage also support this concept. In many respects there is difference in the response against trypsin injection in normal and survived dogs. For example, difference in A. T. A. between the groups of normal and survived animals became extremely remarkable later than 60 minutes after the injection of trypsin. In normal animals, A. T. A. reached nearly zero 100 minutes after the injection, restoring slightly thereafter. This is accepted to suggest that the mechanism of A. T. A. supply begins to work as late as this stage. In survived dogs, it is presumed that ceriain factor somewhat different from those in normal animals are participating in the mechanism of A. T. A. supply, as suggested from gradual increase in A. T. A. and rapid decrease in active trypsin 60 minutes after the injection. Although individual difference was naturally observed more or less in other normal animals, the tendency was constant as a whole.

Blood pressure fell swiftly within a few minutes after the injection of trypsin. Survived dogs almost recovered 150 minutes after the injection, while normal dogs still remain in a state of shock at this period, without any sign of recovery.

It is worth while noticing that there was such a remarkable difference between normal dogs and survived ones despite the injection of trypsin with the same condition. In survived dogs, as shown in the present experiment, once markedly fluctuated active trypsin and trypsin inhibitor 2 weeks after the onset of the disease had restored to preoperative level. As the explanation for this observation, various possibilities can be presumed. Firstly, inhibitor-precursor may be activated by some moment to become the inhibitor and compensate the deficit of it, secondly, trypsin inhibitor or its precursor existing in the tissues may be mobilized to blood stream and thirdly, both of these two may participate in the process. Since precursor of trypsin inhibitor cannot be determined at present, possible existence of trypsin inhibitor in the tissue and its change were investigated in the present experiment. Such possibility can be considered necessarily not only at the injection of trypsin but also in acute pancreatitis. From above mentioned reasons, observations were carried out in the liver and spleen, the results of which are summarized in Fig. 8. Although the changes in trypsin inhibitor in the tissues were different between normal and survived dogs, it cannot be immediately concluded that trypsin inhibitor in the tissues is mobilized into blood stream, from this difference. However, concerning trypsin inhibitor in the tissue in dogs of acute pancreatitis, an interesting results of inverted relationship between the inhibitor in serum and tissues in the initial stage of the disease, as shown in Fig. 9, which is accepted to suggest the possibility of mobilization of trypsin inhibitor in the tissues into blood stream. Particularly, it is considered from the responsive change in A. T. A. in the liver against that in serum at 6th hour that mobilization of trypsin inhibitor occurs more easily from the liver under certain conditions. Such phnomenon, however, may be transient, since the liver meets particularly large amount of liberated trypsin initially owing to its anatomical relation, and it can not be denied to presume that trypsin inhibitor was supplied here contrariwise from the blood stream.

On the other hand, some reports[15)21)] are seen that activity of trypsin inhibitor increases

at tissue destructing lesions. Hence, the possibility that trypsin inhibitor in inactivated type within the normal tissue may be activated by some factors at destruction of the tissue and liberated into the blood stream cannot be ignored.

Although A. T. A. level at exploratory laparotomy fluctuated more or less, the pattern was definitely different from that of acute pancreatitis. Its change being small, results of the present experiment is little influenced by it.

To summarize the above, it is as follows.

Unexpectedly large amount of trypsin is liberated at acute pancreatitis as well as other enzymes, and it is strongly suggested that this liberated trypsin is inactivated first by P. T. I. and further by trypsin inhibitor in blood more strongly, which is represented as A. T. A., leaving slight activity of trypsin. Concerning the toxicity of trypsin, in recent years, toxic substances activated by trypsin is discussed besides trypsin itself, as indirect effect of trypsin.

Pathophysiological significance of trypsin inhibitor is even more great, since it primarily inhibits not only trypsin but these secondary substances. It is a matter of course that besides trypsin inhibitor many factors should be considered to influence the prognosis of acute pancreatitis. It is assumed, however, that activity of trypsin inhibitor may well represents an aspect of degree of defence, and storage or reserve capacity of trypsin inhibitor would be a factor determining the outcome of the disease. This is also confirmed by the observation of RUSH and CLIFFTON[43] that death also occurred in the animals with entire withdrawal of antiproteolytic activity by kaolin injection. Trypsin inhibitor is so much labile due to various conditions such as individuality and others, and there remain some problems for the diagnostic utilization. Concerning the correlation between trypsin and trypsin inhibitor, only the approximate outline could be disclosed by the method of determination employed in the present experiment, since there is no accurate method of determination at present for each property of trypsin inhibitor and trypsin and trypsin inhibitor in compound form. In addition, it is easily presumed that some part of trypsin inhibitor is consumed for the neutralization of other proteolytic enzymes[60], as mentioned in the above, which makes the problem even more complicated in investigation of the correlation between trypsin and trypsin inhibitor.

Concerning the significance of peritoneal fluid, as mentioned repeatedly, its effect of lessening the toxicity by dilution and neutralization should be noticed. Accordingly, operasion modus of merely traditional removal of peritoneal fluid at acute pancreatitis should be further studied.

RUSH and CLIFFTON[43] reported an increase in antiproteolytic activity in patients recovered from acute pancreatitis. In the present experiment also greater antagonistic capacity against newly injected trypsin could be demonstrated in survived dogs, giving an interesting impression as if it were immune state after recovered from some diseases. In the present experiment, immunological study was not carried out, but GROB[19] insisted that the reaction between trypsin and antitrypsin should be considered to be different from antigen-antibody reaction. Any way, this prodlem is particularly interesting in another respect of chronic relapsing pancreatitis.

By examining attitude of trypsin inhibitor in blood and tissues, a possibility of mutual

transfer between these two and, further, complexity of defence mechanism of organism were strongly suggested.

V. SUMMARY

Acute pancreatitis was experimentally produecd in dogs, and active trypsin and trypsin inhibitor were determined. The results obtained were summarized as follows;

1. From a series of experiments, it was strongly suggested that trypsin is liberated at acute pancreatitis hematogenously, lymphogenously and immediately to the surrounding tissues, most of which is shortly inactivated by trypsin inhibitor in the pancreas and blood.

2. Increase in active trypsin and decrease in trypsin inhibitor in blood are more remarkable in the cases of serious development of the disease compared with those of mild one.

3. Attitude of active trypsin and trypsin inhibitor in peritoneal fluid was somewhat different from that of blood and significance of peritoneal fluid as buffer for trypsin should be recognized.

4. Possibility of mutual transfer between trypsin inhibitor in blood and tissues was suggested.

5. Higher resistance against trypsin injection was observed in dogs once survived acute pancreatitis than in normal dogs.

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(*in Japanese)

和文抄録

急性膵炎に於けるTrypsin Inhibitorの推移

金沢大学医学部第2外科教室（主任 本庄一夫教授）

小 幡 一 雄

急性膵炎に際し，他酵素の如くに血中 Trypsin が上昇しない現象の一因として，生体内 Trypsin Inhibitor による不活性化の問題が考えられる．著者はこの可能性を確かめ，又 Trypsin Inhibitor と逸脱 Trypsin の相互関係を明らかにするため，犬の膵管内に自家胆汁を注入して実験的に急性膵炎を作成した上，活性 Trypsin 及び Trypsin Inhibitor を測定し次の知見を得た．

1) 諸種の実験結果，膵炎時 Trypsin も種々の形で膵より逸脱するが，これらの大部分は膵 Inhibitor 並びに血中 Inhibitor により短時間で不活性化される事が強く暗示される．

2) 膵炎の中，重症化を来たすものは軽症で経過するものに比べ血中活性 Trypsin の上昇並びに血中 Trypsin Inhibitor の減少が著明である．

3) 腹腔滲出液中の活性 Trypsin, Trypsin Inhibitor の消長は血中の夫れとは多少異なつた態度をとり，滲出液の Trypsin 緩衝作用に意義を附すべきである．

4) 血中 Trypsin Inhibitor と組織中 Trypsin Inhibitor の相互移行の可能性が考えられる．

5) Trypsin 静注に対し，一度膵炎を経過せる犬は正常犬よりも Trypsin 侵襲に抵抗性の高いのが証明された．